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Genitourinary Cancer

Molecular Subtypes Improve Prognostic Value of International Metastatic Renal Cell Carcinoma Database Consortium Prognostic Model

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Abstract

Introduction. Gene-expression signatures for prognosis have been reported in localized renal cell carcinoma (RCC). The aim of this study was to test the predictive power of two different signatures, ClearCode34, a 34-gene signature model [Eur Urol 2014;66:77–84], and an 8-gene signature model [Eur Urol 2015;67:17–20], in the setting of systemic therapy for metastatic disease.

Materials and Methods. Metastatic RCC (mRCC) patients from five institutions who were part of TCGA were identified and clinical data were retrieved. We trained and implemented each gene model as described by the original study. The latter was demonstrated by faithful regeneration of a figure and results from the original study. mRCC patients were dichotomized to good or poor prognostic risk groups using each gene model. Cox proportional hazard regression and concordance index (C-Index) analysis were used to investigate an association between each prognostic risk model and overall survival (OS) from first-line therapy.

Results. Overall, 54 patients were included in the final analysis. The primary endpoint was OS. Applying the ClearCode34 model, median survival for the low-risk—ccA ($n = 17$)—and the high-risk—ccB ($n = 37$)—subtypes were 27.6 and 22.3 months (hazard ratio (HR): 2.33; $p = .039$), respectively. ClearCode34 ccA/ccB and International Metastatic Renal Cell Carcinoma Database Consortium (IMDC) classifications appear to represent distinct risk criteria in mRCC, and we observed no significant overlap in classification ($p > .05$, chi-square test). On multivariable analyses and adjusting for IMDC groups, ccB remained independently associated with a worse OS ($p = .044$); the joint model of ccA/ccB and IMDC was significantly more accurate in predicting OS than a model with IMDC alone ($p = .045$, F-test). This was also observed in C-Index analysis; a model with both ccA and ccB subtypes had higher accuracy (C-Index 0.63, 95% confidence interval [CI] = 0.51–0.75) and 95% CIs of the C-Index that did not include the null value of 0.5 in contrast to a model with IMDC alone (0.60, CI = 0.47–0.72). The 8-gene signature molecular subtype model was a weak but insignificant predictor of survival in this cohort ($p = .13$). A model that included both the 8-gene signature and IMDC (C-Index 0.62, CI = 0.49–0.76) was more prognostic than IMDC alone but did not reach significance, as the 95% CI included the null value of 0.5. These two genomic signatures share no genes in common and are enriched in different biological pathways. The ClearCode34 included genes *ARNT* and *EPAS1* (also known as HIF2a), which are involved in regulation of gene expression by hypoxia-inducible factor.

Conclusion. The ClearCode34 but not the 8-gene molecular model improved the prognostic predictive power of the IMDC model in this cohort of 54 patients with metastatic clear cell RCC. ***The Oncologist*** 2017;22:286–292

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Implications for Practice: The clinical and laboratory factors included in the International Metastatic Renal Cell Carcinoma Database Consortium model provide prognostic information in metastatic renal cell carcinoma (mRCC). The present study shows that genomic signatures, originally validated in localized RCC, may add further complementary prognostic information in the metastatic setting. This study may provide new insights into the molecular basis of certain mRCC subgroups. The integration of clinical and molecular data has the potential to redefine mRCC classification, enhance the understanding of mRCC biology, and potentially predict response to treatment in the future.

Introduction

Gene-expression signatures for prognosis have been reported in localized renal cell carcinoma. The objective of the present study was to test the predictive power of two different signatures, ClearCode34 (a 34-gene signature model [1]) and an 8-gene signature model [2], in the setting of systemic therapy for metastatic disease. Renal cell carcinoma (RCC) has a variable natural history in terms of aggressiveness for which there are few validated genomic prognostic markers [3, 4]. Prognostic classification and risk assessment models have been based on pathologic variables to predict individual patient prognosis. Commonly used algorithms to assess the risk of disease recurrence after surgery for localized disease include the Stage Size Grade and Necrosis score [5] and the UCLA Integrated Staging System [6]. In advanced disease, Memorial Sloan Kettering Cancer Center (MSKCC) and International Metastatic Renal Cell Carcinoma Database Consortium (IMDC) models are commonly used for prognostic stratification of patients with metastatic RCC (mRCC) [7, 8].

Global gene expression profiling has assisted to define subtypes of cancer [9]. Advances in sequencing technologies and collaborative genome projects are providing researchers with massive lists of gene associations [10]. Indeed, The Cancer Genome Atlas (TCGA) project has already provided deep molecular knowledge in many cancer types, including RCC [3]. However, the potential utility of these data remains largely unknown, and change in the clinical practice has yet to be accomplished [11]. Since the first gene expression profiling study described in RCC [12], several gene signatures have successfully been developed that may predict outcomes in localized RCC, often by dichotomizing (low/high risk of relapse) the targeted population [1, 2, 12–14].

ClearCode34 is a validated gene expression signature that measures the expression levels of 34 genes in a surgically resected primary kidney cancer sample to classify a tumor as one of two intrinsic subtypes (ccA and ccB), which have been shown to be prognostic in localized patient populations, with ccB type predicting for a worse outcome [1]. The 8-gene signature measures the expression levels of eight genes and classifies clear cell RCC (ccRCC) into two clinical prognostic subtypes. This multigene assay was validated in non-metastatic RCC and in a cohort of mRCC patients was associated with response to targeted therapy [1, 2]. The overall goal of this study aimed to explore the applicability of these signatures in a cohort of mRCC from the TCGA and to see if a model of genomic signature improved the prognostic performance of the IMDC or MSKCC classifications.

Materials and Methods

Tumor Samples and Patients

In total, 57 mRCC patients from five institutions (Dana-Farber Cancer Institute, MD Anderson Cancer Center, University of Pittsburgh Cancer Center, Memorial Sloan-Kettering Cancer Center, and University of North Carolina) who were part of the TCGA ccRCC cohort and treated with targeted therapy were identified. Clinical and pathologic information was collected from the medical records, including treatments received and overall survival (OS). Of the 57 patients identified, 54 patients with both available RNAseq gene expression data and outcome information were finally included in survival analysis. Institutional review board approval was obtained locally.

The Cancer Genome Atlas Data

Open and public TCGA (http://cancergenome.nih.gov/) data repositories were our primary source of metadata. We examined several genomic signatures of which two gene signature passed our criteria of size, feasibility and ability to reproduce the models (Fig. 1). The R code to reproduce the analyses performed are provided in the supplemental online Appendix.

Model Training of the ClearCode34 Gene Signature

The ClearCode34 gene signature model was trained as described by Brooks et al. [1]. The 34-gene signature model was trained on gene expression profiles of 40 tumors, of which 23 were ccA and 17 were ccB cases using the nearest centroid classifier (PAM) algorithm using the pamr package in R [13]. R code and training data were provided by the study authors [13].

We performed two tests to verify we had faithfully implemented the gene model. First, we predicted the ClearCode34 subtype of 153 tumors (UNC cohort) described in supplementary Table 4 from Brooks et al. [1]. We correctly assigned all tumors (ccA $n = 67$; ccB $n = 86$) with probability of subtypes identical to those previously reported [13], verifying we had successfully replicated the model.

Secondly, we predicted the ClearCode34 subtypes of 380 tumors from The Cancer Genome Atlas Kidney Renal Clear Cell Carcinoma (TCGA KIRC) study that had been also classified by Brooks et al. [1]. The published classification for these tumors was available in supplementary file 3 [1]. When we classified the 380 tumors, we observed that the accuracy of prediction results was dependent on data preprocessing. Data must be log transformed, row median centered, and scaled. Without row (gene) median centering, accuracy was reduced to 67%. Scaling using the row (gene) medians of the 380 tumors resulted in 14 misclassified samples (96% accuracy). The gene (row) median is dependent on the subset of tumor profiles or, more specifically, on proportion of ccA and ccB patients in the study (see supplemental methods file). Therefore, TCGA tumors were scaled using a constant median scaling factor (median gene expression of the 533 patients' cohort). Most tumors

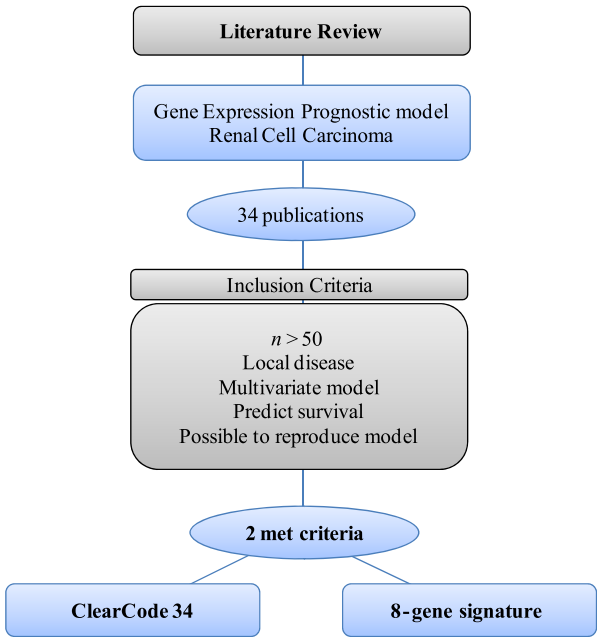

Figure 1. Flow chart shows selection of signatures.

(304/308) were correctly classified using this scaling, resulting in accuracy of 99% (4 ccA samples were misclassified as ccB).

Model Training of the 8-Gene Signature

The model coefficients of the eight genes provided in supplementary Table 7 [2] were used to derive a class-based outcome variable for Cox proportional hazards analysis. To validate the model, we reproduced Figure 2b, which presented results of analysis of TCGA tumors ($n = 419$). The authors did not provide this list of 419 samples. In order to reproduce the analysis as closely as possible, we filtered TCGA data to those cases ($n = 470$) with clinical annotation on October 2, 2013, as this is the download date given in the supplement of the article, and to those cases that were part of the TCGA publication (*Nature*, Volume 499 Number 7456, July 4, 2013), which created a list of 418 cases (supplemental online Fig. 1 [3]). The relative risk of 418 cases was predicted using the Cox linear predictors of the 8-gene expression signature, which was stratified at zero, generating two groups of patients. In agreement with Choudhury et al. [2], the smaller group ($n = 150$) was associated with poor survival (hazard ratio (HR): 2.52; $p = 2.9 \times 10^{-8}$), and 8-gene signature was significantly associated with OS of TCGA KIRC patients.

Descriptive and Survival Analysis

Continuous variables were described with the median and range values. OS was calculated from the beginning of first-line targeted therapy to death of any cause. Cox proportional hazard models and likelihood ratio using OS were used to compare competing survival models. Uno's version of the concordance index (C-Index) was used to predict the final risk model. The C-Index is conceptually similar to receiver operating characteristic curve analysis and ranges from 0 to 1, where 0.5 is null (random, no discrimination). To be statistically significant, the C-Index should have a 95% confidence interval (CI) not including 0.5. We calculated C-Index point estimates and 95% CIs using the *survC1* R package v1.0-2 with truncation time Tau = 3 years. Therefore, the C-Index should be interpreted as having predictive value for events that occur in 0–3 years.

Analysis of Gene Expression of Signatures in the mRCC

The expression profile of genes in each gene signature was also visualized using hierarchical cluster analysis using Pearson correlation coefficient distance with average linkage clustering. Gene set enrichment analysis (GSA) of gene signatures was performed using the Bioconductor/R libraries DOSE, reactomePA (function enrichPathway) and clusterprofiler (enrichGO) to test if the gene signatures were enriched in genes in Reactome (R package graphite_1.15.1, 1526 pathways) or Gene Ontology (GO, GO.db_3.1.2) gene sets biological process (BP), molecular function (MF) and cellular component. *p* values were adjusted for multiple testing by controlling the False Discovery rate, also called the Benjamini and Hochberg correction [15]. Only gene sets or pathways with minimum size of 4 and maximum size of 500 (default setting) were studied. The default minimum gene set size is ten, and this was modified given the small number of genes in the ClearCode and C8 signatures

Results

Patient Characteristics

Overall, 54 patients were included in the analysis. The main clinical and demographic characteristics of the patients are summarized in Table 1. The distribution of metastases was as expected in a conventional mRCC cohort; with the lung, lymph node, and bone being the most common sites of metastases. The proportions of patients in IMDC prognostic risk groups were 15%, 65%, and 20% for good, intermediate, and poor risk, respectively.

Comparison of the Genes in Both Signatures

There was no gene overlap in genes in the 34-gene ClearCode34 and 8-gene Choudhury signature. GSA suggested that there was no functional or pathway overlap in the gene signatures. Genes in the 8-gene signature were not enriched in any Reactome pathway or GO BP but were enriched in MF "receptor binding" (adjusted $p < .05$).

By contrast, the 34 genes in ClearCode34 signature were significantly enriched (adjusted $p < .05$) in two Reactome pathways and nine GO MF terms. The enriched Reactome pathways were "Regulation of gene expression by Hypoxia-inducible Factor" (adjusted *p* value <.05) and "Bicarbonate transporters" (adjusted *p* value <.05), as these pathways contain the ClearCode34 genes *EPAS1* and *ARNT* and *SLC4A4* and *SLC4A3*, respectively. The nine MF GO terms included "primary amine oxidase activity" (adjusted $p < .01$), several MF-related inorganic anion exchanger activity (adjusted *p* value <.05), Wnt-protein binding, and Frizzled binding (adjusted $p < .05$; supplemental methods file).

When we compared the top 100 pathways and gene sets that were highly ranked but not significant (unadjusted *p* value <.05), we found no significant overlap in ClearCode34 and the 8-gene Reactome pathways or GO terms. Whilst not significant, ClearCode34 but not the 8-genes signature included one gene (*PRKAA2*) that is involved in mammalian target of rapamycin (mTOR) signaling.

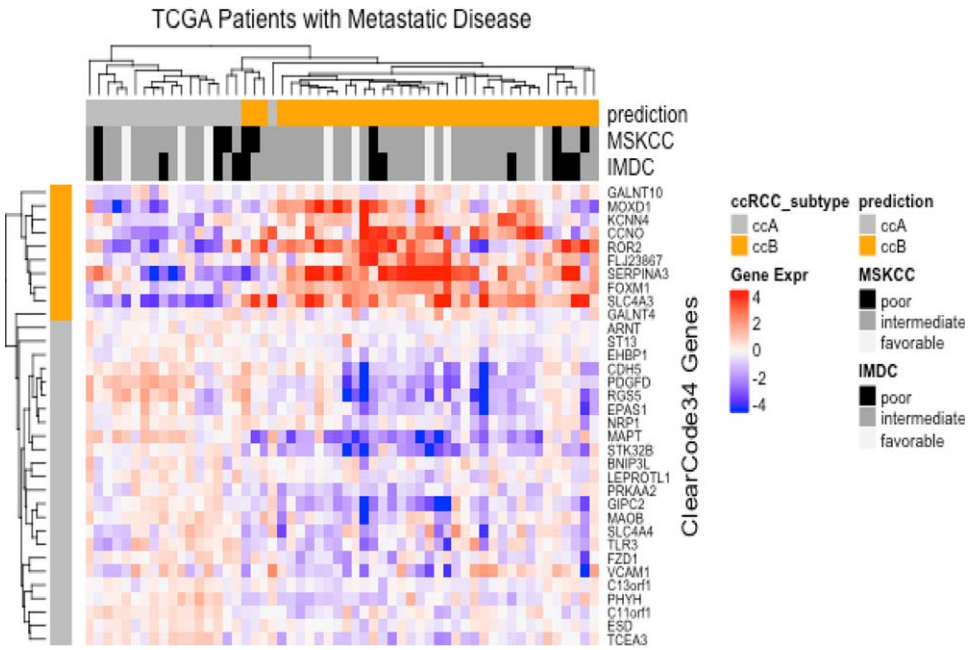

Figure 2. Heat map showing hierarchical clustering analysis of the gene expression profiles of the ClearCode34 genes in metastatic renal cell carcinoma TCGA tumors ($n = 54$). Most tumors ($n = 37$) were classified as ccB. There was no significant overlap in the ccA/ccB subtype classification and the MSKCC or IDMC risk class.

Abbreviations: IMDC, International Metastatic Renal Cell Carcinoma Database Consortium; MSKCC, Memorial Sloan Kettering Cancer Center; TCGA, The Cancer Genome Atlas.

OS—ClearCode34

ClearCode34 stratified mRCC patients into two groups (Fig. 2): ccA ($n = 17$) and ccB ($n = 37$) with a median OS of 27.6 and 22.3 months, respectively. The signature was distinct from the IMDC and MSKCC clinical classification (Pearson's chi-square test, $p > .05$), confirming that the gene signature represents a different substratification of the patients (Fig. 2).

Patients in the ccB group had significantly worse OS in univariate Cox proportional-hazards analysis (HR: 2.33; 95% CI, 1.02–5.31; $p = .039$; Fig. 3A). In multivariable analysis, adjusted for either the MSKCC or IMDC groups, ccB remained independently associated with a worse OS (likelihood ratio (LR) test $p = .025$ and $p = .044$, respectively).

The C-Index of ccA/ccB, MSKCC, or IMDC was 0.57, 0.58, and 0.60, respectively, but in each model, the 95% CIs include the null model 0.5. The accuracy of both the IMDC and MSKCC models was more accurate when the ClearCode34 molecular subtype was added to the model. A multivariate model of ccA/ccB subtype and IMDC or MSKCC risk score had a Uno's C-Index of 0.63 (CI = 0.51–0.75), and the CIs did not span the null model, indicating that the multivariate model was a more accurate predictor of outcome than IMDC or MSKCC alone (Fig. 3B).

OS—8-Gene Signature

The 8-gene signature stratified mRCC patients into a larger group of poorer prognosis patients ($n = 31$) and a smaller group with favorable prognosis ($n = 23$), with a median OS of 27.6 and 22.3 months, respectively. Both signatures predicted 16 patients as good prognosis and 29 patients as poor prognosis, and this overlap was significant (Pearson's chi-square test with Yates' continuity correction, $p < .0001$). The poor and good prognosis 8-gene groups and ClearCode34 classification provided discordant predictions in 20% ($n = 11/54$) of patients. The tumor classification model of the 8-gene signature was not significantly associated with worse OS (HR 1.68, CI 0.846–3.32; $p = .134$; Fig. 4). Additionally, whilst a multivariate model of the 8-gene classification and either IMDC (C-Index = 0.62, CI = 0.49–0.76) or MSKCC (C-Index = 0.62, CI = 0.49–0.75) improved prediction accuracy over IMDC or MSKCC alone, it did not reach significance in this cohort of 54 cases, as the CIs of C-Index of the multivariate model included the null value (0.5).

Discussion

The established methods for risk stratification of mRCC patients treated with targeted therapy rely on clinical factors, which defined three subgroups (favorable, intermediate, and poor). However, interpatient variability within these clinical subgroups is high. Here, we sought to improve characterization of mRCC subgroups by adding genomic classification. In contrast to classic methods, we evaluated patient survival prediction in a metastatic cohort from the TCGA using different molecular signatures that were developed in primary RCC tumors. This approach enables investigating the clinical utility of available large-scale genomic data.

To this end, we studied two genomic signatures in mRCC patients treated with targeted therapy. We were able to successfully reproduce published results from the ClearCode34 and the 8-gene signatures with an accuracy of nearly 99%, which is notable and is inconsistent with reports of poor reproducibility in genomic studies [16]. Whilst this study has limitations (discussed below), we provide evidence that molecular and genomics biomarkers have potential in clinical decision making in mRCC. Both gene expression signatures that we tested stratified mRCC into groups that were significantly distinct from traditional clinical risk groups (IMDC, MSKCC).

Table 1. Patient baseline characteristics of the mRCC cohort

Characteristic	No.	%
Sex		
Male	37	66.6
Female	17	33.6
Age		
Median	62	
Range	39–84	
MSKCC Criteria		
Good	8	14.8
Intermediate	38	70.3
Poor	8	14.8
IMDC Criteria		
Good	8	14.8
Intermediate	35	64.8
Poor	11	20.3
No. Metastases		
1	20	37
2	14	26
>2	20	37
Location Metastases		
Lung	42	77.7
Lymph node	24	44.4
Liver	3	5.5
Bone	20	37.0
Brain	12	22.2
VEGF-targeted first- line therapy	52	92.8
Sunitinib	26	52.0
Pazopanib	7	12.9
Axitininb	1	1.8
Sorafenib	8	14.8
Bevacizumab	8	14.8
mTOR inhibitor	4	7.4
Temsirolimus	4	7.4

Abbreviations: IMDC, International Metastatic Renal Cell Carcinoma Database Consortium; MSKCC, Memorial Sloan Kettering Cancer Center; mTOR, mammalian target of rapamycin; VEGF, vascular endothelial growth factor.

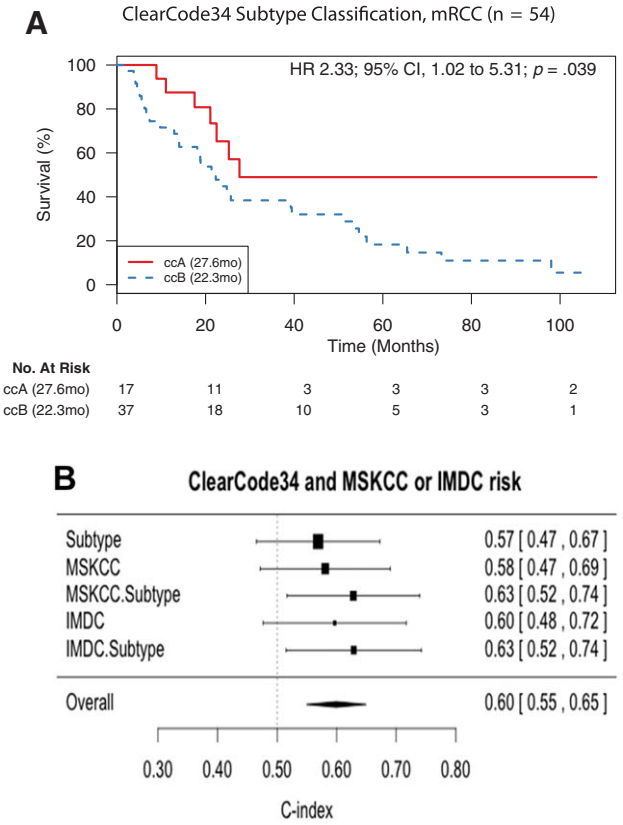

Figure 3. mRCC tumors stratified by the ClearCode34 signature were associated with overall survival. **(A):** Kaplan–Meier plot of overall survival of metastatic renal cell carcinoma Cancer Genome Atlas tumors, which were stratified into two molecular subtypes using ClearCode34 gene signature. **(B):** Forest plot shows the C-Index of the ClearCode34 subtype, MSKCC or IMDC risk models, alone or joint model of ClearCode34 subtype and MSKCC or IMDCC. The C-Index is conceptually similar to receiver operating characteristic curve analysis and ranges from 0 to1 where 0.5 is null (random, no discrimination). To be statistically significant, the C-Index should have a 95% confidence interval not including 0.5. The C-Index is indicated by a square, and the whiskers represent the 5% and 95% quartiles.

Abbreviations: CI, confidence interval; C-Index, concordance index; HR, hazard ratio; IMDC, International Metastatic Renal Cell Carcinoma Database Consortium; MSKCC, Memorial Sloan Kettering Cancer Center.

Despite the fact that the 8-gene signatures did not validate, it was weakly prognostic in this small cohort of patients. By contrast, the ClearCode34 subtypes had significant prognostic predictive power in this cohort of 54 mRCC patients. It stratified patients into a good and poor prognosis groups that had significantly different survival profiles, and this difference remained significant even when adjusted for traditional risk-associated clinical variables (IMDC).

Gene-based prognostic markers have been translated into clinical practice in few diseases, most notably in breast cancer [16], which is an important step toward personalized medicine. Whilst, the clinical application of genomic biomarkers has yet to be established in mRCC, in this study, we demonstrate that a genomic signature, ClearCode34, can stratify patients into subgroups in mRCC. A joint model of molecular subtypes and clinical factors improved the accuracy of prognostic prediction beyond the clinical factors (IMDC or MSKC). This is a significant finding, as it suggests that genomics may augment clinical data to guide better treatment strategies for patients in the metastatic setting.

We examined the 34 genes in the ClearCode34 signature to discover a possible biological basis for its prognostics potential in mRCC. The ClearCode34 genes include one gene that is associated with mTOR signaling (*PRKAA2*) and two genes (*EPAS1*, *ARNT*) that are involved in the "Regulation of gene expression by Hypoxia-inducible Factor" [17]. We tested all 18,502 genes in the TCGA to discover genes prognostic in mRCC, but neither *EPAS1* nor *ARNT* were significant predictors of prognosis in mRCC. ClearCode34 also included the genes *SLC4A4* and *SLC4A3*, which are significantly enriched in genes in the pathways "Bicarbonate transporters." Gene expression of *SLC4A3* was significantly associated with worse outcome (HR: 3.39,

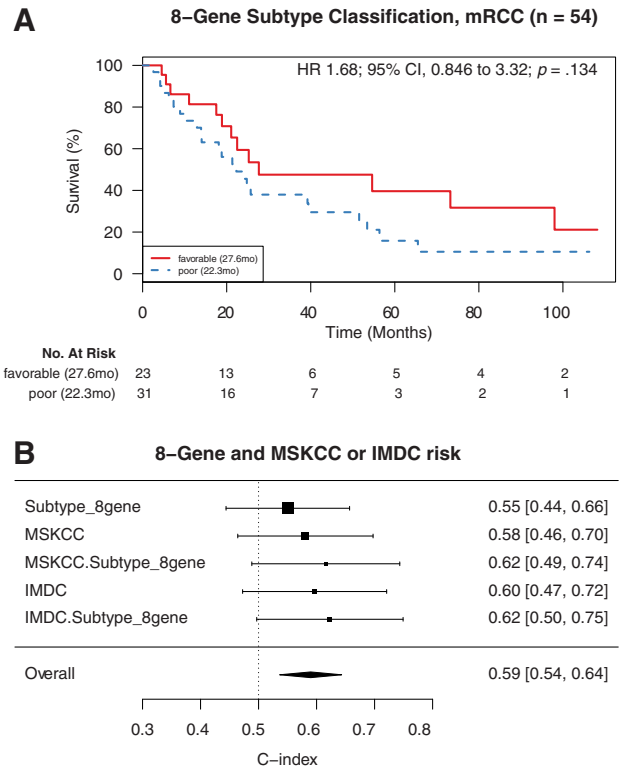

Figure 4. Subtypes stratified by the 8-gene signature were not significantly associated with overall survival. **(A):** Kaplan–Meier plot of overall survival of mRCC TCGA tumors, which were stratified into two molecular subtypes using 8-gene model. **(B):** Forest plot shows the C-Index of the 8-gene, MSKCC or IMDC risk models, alone or joint model of ClearCode34 and MSKCC or IMDCC. The C-Index is conceptually similar to receiver operating characteristic curve analysis and ranges from 0 to1 where 0.5 is null (random, no discrimination). To be statistically significant, the C-Index should have a 95% confidence interval not including 0.5. The C-Index is indicated by a square and the whiskers represent the 5% and 95% quartiles.

Abbreviations: CI, confidence interval; C-Index, concordance index; HR, hazard ratio; IMDC, International Metastatic Renal Cell Carcinoma Database Consortium; MSKCC, Memorial Sloan Kettering Cancer Center.

95% CI 1.63–7.04, unadjusted $p < .001$) in our cohort of 54 mRCC tumors and was the only gene in the ClearCode34 signature that was significantly associated with worse outcome (unadjusted p values $<.05$). The genes *MAOB*, *CDH5*, *PDGFD*, *GIPC2*, and *RGS5* were marginally associated with better outcome ($p < .05$). Bicarbonate transporters play central roles in pH regulation and thus have been implicated in cancer pathogenesis pathways, including the metabolic shift in most cancer cells toward more acid-producing pathways and pH changes which are associated with hypoxia development in poorly perfused regions of the tumors [18].

Our data should be interpreted cautiously, as this study has a number of limitations. First, only 54 tumors were studied. This is a small sample size with limited prognostic power. Second, we report that the subtypes predicted by the ClearCode34 were sensitive to data preprocessing. The published ClearCode34 algorithm expects that gene expression values are median centered, and we observed variability in the gene median values depending on the subset of patients studied. This is to be anticipated, as it has been previously reported that gene signatures based on normalizing test data may be irreproducible if the cohort changes composition or size [19]. Third, we generated a constant reference scaling; however, we have not tested if this can be applied in independent datasets. Heterogeneity is now understood to be one of the central hallmarks of cancer [20], so one concern for any TCGA study is that only a single sample for each tumor was included [3]. Also, there is always a concern if a batch effect or a sampling artifact can direct to misleading results in high throughput genomic analyses. Most importantly, due to the small sample size, study of larger metastatic cohorts is required to validate the model in advanced RCC, appreciate the genomic differences between subgroups, and confirm the utility of genomic signature in mRCC.

Our hypothesis arises from the concept that prognosis of metastatic disease is at least partially driven by the biology of the primary tumor. But can models derived from primary renal tumors be applied in the metastatic setting? The interactions between the primary tumor and metastasis have been extensively studied in RCC [21]. The evidence suggests that multiple and complex interactions occur between the primary tumor and metastatic sites. However, several arguments such as spontaneous regression of metastasis after nephrectomy support that, in RCC, prognosis is determined by the original tumor [22]. Studies have also shown that although metastatic signatures are developed gradually during tumor progression; the main fraction of the signatures was already present in the primary tumor with not much difference of expression levels between primary and metastatic tumors [23]. Moreover, other studies have identified metastasis-related genes in primary ccRCC tumors as potential biomarkers to differentiate the tumor prognosis [24]. mRCC is entering an era of expanding therapeutic approaches, and patients will have a considerable number of options, including vascular endothelial growth factor-targeted therapy, mTOR inhibitors, and immunotherapy, such as PD-1/PD-L1 inhibitors. The differential characteristics between subgroups linked to each of the expression signatures eventually may help guide patient management. This study suggests that genomic signatures have value in the metastatic setting, and further investigation is warranted.

Conclusion

These data suggest that genomic signatures from primary tumors can have a prognostic role in the metastatic setting. We validated the ClearCode34 but not the 8-gene molecular signature model, as prognostic for survival in patients with mRCC treated with targeted therapy.

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Disclosures

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